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New Type of Antibody-Enzyme Conjugate Which Specifically Kills *Candida albicans*

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A new type of antibody-enzyme conjugate was made, and its possible application to *Candida* infection was studied. Both lactoperoxidase and xanthine oxidase were conjugated to specific antibody against *Candida albicans*. In vitro microbiocidal activity of the new antibody-enzyme conjugate, when incubated together with xanthine and a minute amount of halides, showed a remarkable level of candidacidal ability. When the new antibody-enzyme conjugate was given to *Candida*-infected mice, followed by injecting xanthine and a minute amount of halides, about 50% of these heavily infected mice survived, whereas all control nontreated mice died. These data suggest that the further elaboration of this new antibody-enzyme conjugate might lead us to improve our therapeutic methods of clinical medicine.

Klebanoff et al. (1) proposed a potent antimicrobial and antitumor agent composed of myeloperoxidase, halides (I, Br, Cl), and a minute amount of hydrogen peroxide. The activity of myeloperoxidase can be substituted by lactoperoxidase and horseradish peroxidase. Knowles et al. (2) have reported that the conjugate of specific antibody and glucose oxidase showed a remarkable in vitro bactericidal effect against *Escherichia coli*. We are now planning to make a new type of antitumor agent composed of lactoperoxidase, xanthine oxidase, and anti-tumor antibody. So, the antibody specifically escorts the lactoperoxidase and xanthine oxidase to the target cells. After the excess or unconjugated agents were excreted from the body, xanthine and a minute amount of KI were injected in it. Thus, the target cells are specifically damaged by the combined effects of these factors. We call this a "new antibody-enzyme conjugate" (new AEC). In the present investigation, we used *Candida albicans* as a target cell because of its strong antigenicity and the lack of good antifungal drugs.

After *C. albicans* ATCC 401 and *Sporotrichum shenkii* (isolated strain) were treated with 1% Formalin, they were injected six times into the rabbits intravenously. The specific antibody was separated by ammonium sulfate fractionation. The conjugation of the antibody to lactoperoxidase and xanthine oxidase was performed by Knowles' method (2). The final biological activity of 1 mg of the new AEC consisted of 0.03 IU (3) of xanthine oxidase, 0.3 pyrogallol unit of lactoperoxidase, and an antibody titer of 64 to 256 by the fungal agglutination test. Table 1 shows the reaction to each component of the antimicrobial agents against the specified target cells. Each component of antimicrobial agents is reacted with each target cell at 37°C for specified times, followed by a viable cell count of the target cell by colony formation. Whereas the mixture of the lactoperoxidase, xanthine oxidase, xanthine, and KI had a strong fungicidal activity, one of these components or antibody alone showed no fungicidal activity. The new AEC displayed a specific fungicidal activity against *C. albicans* and *S. schenkii*. Three other separate experiments showed quite similar inclinations. These results suggest that the new AEC therapy has a strong fungicidal activity in vitro.

Next, experiments were performed to observe whether new AEC has a specific fungicidal activity in vivo. Intravenous injections of 10^7 cells of *Candida albicans* ATCC 401 were given to male mice (C3H/He) 6 weeks of age. After 5 days, 0.5 mg of the new AEC described in the preceding section was injected intraperitoneally into each mouse. A period of 6 h was allowed to lapse to clear the unconjugated AEC; then 10 mM of KI and 1 mg of xanthine were injected intraperitoneally into each mouse. After 14 days, the number of surviving *Candida*-infected mice was counted. As shown in Table 2, 33% of the infected mice treated with the new AEC were dead. The percentages of dead mice after treatment with lactoperoxidase and KI were 87 and 80%, respectively. Figure 1 shows the results of

TABLE 1. *In vitro fungicidal activity of the new AEC*[a]

Target	New AEC	Antibody	Xanthine	Lactoperoxidase	KI	H_2O_2	Viable cell count (mean ± SD) 0 min	30 min	90 min
C. albicans ATCC 401	–	–	–	–	–	–	5.2×10^6	$(4.9 \pm 0.7) \times 10^6$	$(3.8 \pm 0.5) \times 10^6$
	–	–	–	+	+	+	5.2×10^6	$(2.7 \pm 0.6) \times 10^4$	$(3.9 \pm 0.6) \times 10^3$
	–	–	+	–	+	–	5.2×10^6	$(5.3 \pm 1.1) \times 10^6$	$(3.5 \pm 0.1) \times 10^6$
	–	1:25 AbC[b]	+	–	+	–	5.2×10^6	$(2.4 \pm 0.4) \times 10^6$	$(1.2 \pm 0.2) \times 10^6$
	–	1:100 AbC	+	–	+	–	5.2×10^6	$(4.5 \pm 0.5) \times 10^6$	$(2.8 \pm 0.6) \times 10^6$
	1:5 MC	–	+	–	+	–	5.2×10^6	$(6.7 \pm 2.1) \times 10^4$	$(4.3 \pm 1.0) \times 10^2$
	1:25 MC	–	+	–	–	–	5.2×10^6	$(3.9 \pm 0.3) \times 10^6$	$(5.4 \pm 0.5) \times 10^6$
	1:25 MC	–	+	–	+	–	5.2×10^6	$(5.9 \pm 0.7) \times 10^4$	$(8.1 \pm 2.1) \times 10^2$
	1:25 MC	–	–	–	+	–	5.2×10^6	$(6.7 \pm 1.0 \times 10^6$	$(4.4 \pm 0.6) \times 10^6$
	1:100 MC	–	+	–	+	–	5.2×10^6	$(7.2 \pm 0.2) \times 10^4$	$(1.7 \pm 0.3) \times 10^3$
	1:5 MS	–	+	–	+	–	5.2×10^6	$(1.3 \pm 0.1) \times 10^6$	$(9.5 \pm 2.6) \times 10^5$
Sporotrichum schenkii	1:25 MS	–	+	–	+	–	4.9×10^6	$(1.6 \pm 0.5) \times 10^4$	$(4.7 \pm 1.5) \times 10^3$
	1:100 MS	–	+	–	+	–	4.9×10^6	$(2.8 \pm 0.2) \times 10^4$	$(9.3 \pm 3.2) \times 10^3$
	1:25 MC	–	+	–	+		4.9×10^6	$(2.3 \pm 0.4) \times 10^6$	$(1.9 \pm 0.2) \times 10^6$

[a] The reaction was performed in a 37°C water bath shaker. One milligram of the new AEC per ml (described in the text) was employed as the original concentration. Abbreviations: AbC, antibody against *Candida albicans*; MC, new AEC against *Candida albicans*, MS, new AEC against *S. schenkii*); SD, standard deviation.

[b] An agglutination titer of 256 was used as the original concentration of antibodies. The final concentration of 1 mg of xanthine per ml, 0.3 pyrogallol units of lactoperoxidase, 2 mM of KI, and 1 mM of H_2O_2 was used. Antibodies and the new AEC represent the final diluted concentration of them. The fungal number represents the colony-forming units of 1 ml.

TABLE 2. *In vivo fungicidal activity of the new AEC by using C3H/HeJ mice*

Group	Treatment	% of dead mice after 14 days
A	1:1 new AEC	33 (5/15)[a]
B	1:5 new AEC	60 (9/15)[b]
C	1:25 new AEC	71 (10/14)
D	Free xanthine oxidase, lactoperoxidase, xanthine, and KI[c]	80 (12/15)
E	Antibody	87 (13/15)
F	Nontreated	93 (14/15)

[a] Statistically significant difference at $P \leq 0.01$.

[b] Statistically significant difference at $P \leq 0.01$.

[c] The enzyme activities of the free xanthine oxidase and lactoperoxidase used in group D were equal to that contained in the 1:1 new AEC.

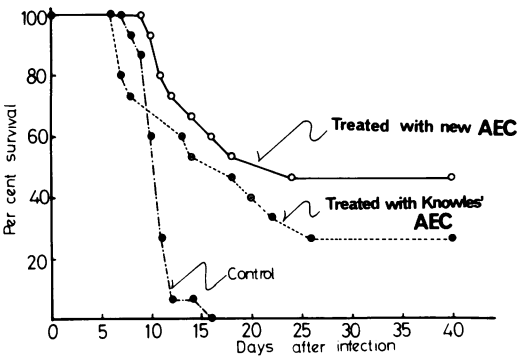

FIG. 1. *Comparison of the time course of the percent survival between the mice treated with the new AEC and those with Knowles AEC. The dose of the new AEC applied in this experiment is equal to that of A in Table 2.*

the time course study of mice by *Candida* infection. Each group consists of 15 mice. The procedure employed for the group of new AEC and control in this figure are equal to those of groups A and F in Table 2. The new AEC employed for this study is the conjugate of antibody and lactoperoxidase by Knowles' method (Knowles AEC [2]). The activity of lactoperoxidase per milligram of Knowles AEC was 0.5 pyrogallol unit, and the antibody titer was 125 by the agglutination test. In this experiment, 0.5 mg of Knowles AEC was injected after 5 days. After 6 h, 0.5 ml of 10 mM KI and 0.5 ml of 10 mM H_2O_2 were injected intraperitoneally, and the percent survival of the mice was observed during 40 days.

As shown in the figure, the treatment by the new AEC as well as the Knowles AEC displayed therapeutic effects on mice candidiasis. The injection of H_2O_2, however, decreased survival of mice, a result of the catalytic activity of some serum factors. When the H_2O_2 was injected below this amount, the therapeutic activity did not appear. These results suggest that the new AEC has a fungicidal activity in vivo, too.

Moolton et al. (4) proposed the idea that the conjugate of antibody to diphtheria toxin possessed a specific cytotoxic activity of mouse kidney cells in vitro. Parker et al. (5) have proposed that a conjugate of the antibody and glucose oxidase produces a strong cytotoxic activity against HeLa cells in vitro. Experiments on the

activities of these substances in vivo have not yet been reported. The present investigation employs a complicated system to obtain the new AEC that specifically destroys target cells both in vivo and in vitro. The conjugate of the antibody and lactoperoxidase and xanthine oxidase actively affects H_2O_2 production in the vicinity of the target cells because serum catalase rapidly breaks down H_2O_2 into H_2O and O_2, when H_2O_2 is directly applied to healthy animals. The results of our investigation imply that further knowledge of AECs can contribute both to our understanding of selective cytotoxicity and to the development of immunological therapy for human cancer.

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